AR201-13843A

EPA'S HPV CHALLENGE PROGRAM: TIER I SCREENING SIDS DOSSIER FOR STYRENE, AR-METHYL-(VINYL TOLUENE) CAS NO. 25013-15-4

DELTECH CORPORATION
BATON ROUGE, LOUISIANA

PREPARED BY:
Kevin N. Baer, Ph.D.
The University of Louisiana at Monroe

June 28, 2002

CONTENTS

OVERVIEW

The Deltech Corporation hereby submit for review and public comment the test plan for vinyl toluene (VT; presume mixtures of meta- and para- isomers in ~60/40% ratio; CAS NO.: 25013-15-4) under Environmental Protection Agency's (EPA) High Production Volume (HPV) Chemical Challenge Program. It is the intent of Deltech Corporation to use both the existing data on VT in conjunction with data from para-methylstyrene and styrene to adequately fulfill all the Screening Information Data Set (SIDS) endpoints. Specifically, the robust summaries of para-methylstyrene are submitted for vinyl toluene. These data are adequate to fulfill all the requirements of the HPV program without the need for the conduct of any new or additional tests. Furthermore, they follow the principles contained in the letter the EPA sent to all HPV Challenge Program participants on October 14, 1999, in which participants are directed to maximize the use of existing data for scientifically appropriate related chemicals in order to minimize animal testing.

JUSTIFICATION FOR USE OF SURROGATE DATA

A careful review of the metabolism, acute and chronic toxicity data, and relevant developmental and reproductive toxicity data for para-methyl styrene, vinyl toluene (presume mixtures of meta- and para- isomers in ~60/40% ratio) and styrene. In my opinion, there are no biological meaningful differences in developmental and reproductive endpoints. These endpoints were not available for the meta- and ortho- isomers of methyl styrene, but no differences would be expected based on the data for the para-isomer or mixtures of vinyl toluene. Detailed comparisons of available data are below. The basis for these conclusions is as follows:

The main metabolites of meta-, para- and ortho- isomers of methyl styrene are similar to the corresponding styrene metabolites. All available acute and chronic data were similar between the isomers of methyl styrene, vinyl toluene mixtures, and styrene. The available data for styrene appears to be adequate and valid to assess reproductive and developmental toxicity. The reproductive and developmental studies for para-methyl styrene appear to be adequate and valid. Although no reproductive effects were found for vinyl toluene (presume mixtures of meta- and para- isomers in ~60/40% ratio), several developmental toxicity studies were found and effects were similar to styrene and para-methyl styrene.

Styrene

The absorption, distribution and metabolism of styrene in the rat have been extensively studied. The elimination of styrene following inhalation, i.v. or oral administration in aqueous solutions is similar, and follows a dose-dependent, two-compartment mathematical model. Styrene is almost totally metabolized to mandelic acid and phenylglyoxylic acid or p-hydroxymandelic acid. An intermediate route is to styrene-7,8-oxide and conjugation with glutathione. Styrene oxide has been shown to be genotoxic and carcinogenic (mice). Metabolic differences exist between rats and mice. Rats (and humans) metabolize styrene to the oxide at a slower rate than mice and detoxify it more rapidly. However, the complete picture is much more complex, with multiple competing pathways, including conjugations with glucuronide, glutathione, glycine and sulfate. There are multiple isozyme forms of the enzymes of activation (i.e., P450) and inactivation, with differential expression during development and in different organs and different subcellular fractions. A complete reproductive and developmental toxicity risk estimation would require careful consideration of all of these factors for reproductive organs and for the conceptus of experimental animals.

Reproduction

A 3-generation reproduction study was conducted in the rat using concentrations of styrene in drinking water (0, 125 and 250 ppm). No adverse effects were seen. The current data does not suggest that styrene is a specific testicular toxicant.

Developmental Toxicity

At doses not toxic to the mother, substantial increases in resorption rate have been seen in the mouse at 250 ppm and the Chinese hamster at 1000 ppm, but not in the rat or rabbit, with exposure during embryogenesis. With exposure commencing on day 1 of pregnancy, one study found an increased preimplantation loss in rats exposed to 11.8 ppm, but another study using 47 and 165 ppm found no effect. At maternally toxic doses there was a clear increase in resorption rate in the mouse but not the rat. In another study, no increases in gross malformations were seen in the rat or rabbit exposed to 300 or 600 ppm by inhalation or after 180 or 300 mg/kg orally in the rat. Skeletal variants were increased in the rat after 300 or 600 ppm and in the rabbit after 600 ppm, but increases were within historical control ranges. Thus, styrene may be embryolethal at high doses in animals particularly resistant to the general toxic effects of styrene, but it does not appear to be teratogenic. Female rats were exposed to 300, 100 and 0 ppm styrene oxide; 300 ppm was rapidly lethal, 100 ppm showed no effect on female fertility and no increases in malformations were observed in any exposed group. Rabbits exposed to 50, 15 and 0 ppm styrene oxide (inhalation) showed significant maternal toxicity with a dose-dependent increased in the number of litters with resorptions, although the number of postimplantation deaths/litter was not increased. A review of the developmental toxicity of styrene sponsored by the SIRC concluded that there is no evidence of teratogenicity. Combined with the human experience, this suggests that it would be difficult to justify any further testing of styrene for developmental toxicity in experimental animals.

Carcinogenicity

Styrene in drinking water caused cancer in mice but not in rats. Styrene was not carcinogenic in mice or rats when given orally in corn oil, but there were "suggestive" increases in lung tumors in male B6C3F1 mice. Despite conflicting studies in humans and experimental animals, styrene is not a generally recognized human carcinogen.

Genetic effects

Styrene is mutagenic in the Ames test with metabolic activation. Detoxification of a potential mutagen such as stryene oxide depends on the activity of microsomal epoxide hydrase and glutathione-S-transferase.

Misc. Acute Data

Oral LD50 Rat 4920 mg/kg
Inhalation TCLo Hamster 376 ppm
Inhalation LC50 Rat 4-hr 24 g/m3 (100.8 ppm)
Oral LD50 mouse 316 mg/kg
Inhalation LC50 mouse 4-hr 9500 mg/m3
1,300 ppm highest value which caused only irritant effects but no systemic toxicity in 8-hr inhalation
Inhalation LCLo Rabbit 4-hr 4000 ppm

<u>Para-methylstyrene</u>

After ortho-, meta- and para-vinyl toluenes were injected IP into male rats, the main metabolites were similar to the corresponding styrene metabolites and included ethylene glycol, mandelic acid, glyoxylic acid derivatives and N-acetylcysteine and glucuronide conjugates.

Reproduction

No reproductive effects, 200 mg/kg (2 generation, rat-oral)

Developmental

Not a teratogen when administered orally to rats at a dosage level of 600 mg/kg or less. Treatment did not produce a teratogenic response when administered orally to rabbits at a dosage of 150 mg/kg day or less.

Carcinogenicity

NOEL after a lifetime oral ingestion by rats was 50 mg/kg/day in males and 500 mg/kg/day in females. No specific or unique tumors arose as a result of ingestion.

13-week rat oral and inhalation: Oral, no effects at 100 mg/kg; inhalation, no effects at 500 ppm.

Misc. Acute Studies

IP LD50 Rat 2324 mg/kg

Oral LD50 Rat 2255 mg/kg
Oral LD50 mouse 1072 mg/kg
Inhalation LC50 >3,500 ppm
13-week toxicity – no effects at 500 ppm

Vinyl Toluene

Commercial vinyl toluene, also called methyl styrene, is usually a mixture of the meta- and para- isomers in ~60/40% ratio, but often the toxicicological literature does not distinguish between the various forms. The toxicological properties appear to be similar to those of styrene. Vinyl toluene is excreted mainly in urine in the form of metabolites. The major metabolites which have been detected in rats after a single IP dose of vinyl toluene were p-methylmandelic acid, p-methylglyoxylic acid, p-methylebenzoic acid, p-methylbenzoylglycine, p-methylphenylacetylglycine, p-vinylbenzoylglycine, p-methylphenylacetic acid, p-methylphenylacetylglycine, p-vinylbenzoyl acid, and thioethers (N-acetyl-S-(2-(p-tolyl-2-hydroxyethyl)) cysteine and N-acetyl-S-(1-(tolyl)-2-hydroxyethyl)cysteine). A large proportion of the metabolites were as thioethers at low doses of vinyl toluene rather than at high doses (50 mg/kg/body weight vs 350-500 mg/kg/body weight, when given IP). With the exception of p-vinylbenzoic acid and p-vinylbenzoylglycine all other metabolites are formed by cytochrome P450-dependent oxidation of vinyl toluene with vinyltoluene-7,8-oxide (p-methylphenylethylene-7,8-oxide) as an intermediate (Snyder, 1987).

Reproductive effects

Unable to locate references on possible reproductive effects.

Developmental effects

IP in female rats on days 1 to 15 of pregnancy; 3750 mg/kg caused post-implantation mortality and stunted fetus but no teratogenicity. Administration of 250 mg/kg/day to pregnant rats did not produce an increase in birth defects in the offspring in spite of induction of fetal toxicity. Teratogenic in guinea pigs at a dose of 6 ppm for 4 months or 6200 ppm for 1 month.

Genetic effects

Vinyl toluene produced chromosome damage and an increase in sister chromatid exchanges in human lymphocytes in vitro (0.33 to 4 mM). No gene mutations were observed in Ames test with or without metabolic activation. Vinyl toluene did not induce sister chromatid exchanges or chromosomal aberrations in CHO cells with or without S9.

Carcinogenicity

No evidence of carcinogenic activity for male or female rats exposed to 100 or 300 ppm (inhalation; 6 hr/day, 5 days/week for 103 weeks) and no evidence of carcinogenic activity for male or female mice exposed to 10 or 25 ppm on the same schedule.

13-week rat inhalation: exposures of 60, 160, 400, and 1000 ppm – slightly lower body weights and mild nephrotoxicity at 160; no effects at 60.

Misc. Acute Studies

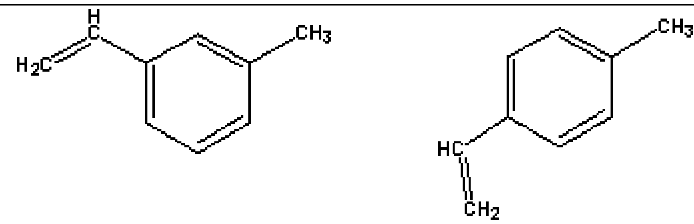
Oral LD50 rat 4900 mg/kg
Oral LD50 mouse 3160 mg/kg
Inhalation LC50 mouse 4-hr 3020 mg/m3

Efforts were made to clarify the molecular basis of styrene toxicity on the dopaminergic systems and to evaluate whether the same mechanism was common to other solvents. Groups of male New Zealand rabbits were exposed to 750 ppm toluene, xylene, styrene, ethylbenzene, vinyl toluene, 7-methyl-styrene, or fresh air (control groups). A significant depletion in both striatal and tubero infundibular dopamine was caused by styrene, ethylbenzene, and vinyl toluene. Methylation of the aromatic ring of styrene did not change its activity, whereas methylation of the side chain drastically reduced its effect on dopamine. Treatment carried out with the main metabolites of aromatic solvents indicated that acidic metabolites of some solvents caused striatal and tubero infundibular dopamine depletion. Present data suggested a chemical reaction between dopamine and some acidic metabolites. The active metabolites have an alpha-keto as the side chain or as a part of their molecule. These keto acids condense nonenzymatically with dopamine (Mutti, 1988).

In conclusion, there are uncertainties that must be recognized. For example, a complete reproductive and developmental toxicity risk estimation would require careful consideration of several factors for reproductive organs and for the conceptus of experimental animals. There are multiple competing pathways for metabolism including conjugation with glucuronide, glutathione, glycine and sulfate. There are also multiple isozyme forms of the enzymes for activation and inactivation, with differential expression during development and in different organs and different subcellular fractions. A complete metabolic picture is not available with styrene or vinyl toluene isomers or with any other industrial chemical. Nevertheless, the weight of evidence strongly suggests that any differences in metabolism or toxicity between the individual isomers or mixtures of vinyl toluene would be unexpected and very unlikely. Therefore, the use of para-methylstyrene and/or styrene data as a surrogate for vinyl toluene is acceptable. The robust summary of para-methylstyrene is submitted with this test plan.

SIDS PROFILE
HPV Test Plan: Part A

DATE: June 28, 2002

1.01A	CAS NO.	25013-15-4
1.01C	CHEMICAL NAME	Styrene, ar-methyl- (Vinyl toluene, mixed isomers)
1.01D	CAS DESCRIPTOR	Not applicable
1.01G	STRUCTURE AND FORMULA	 meta isomer para isomer C_9H_{10}

TEST PLAN JUSTIFICATION/ ISSUES FOR DISCUSSION	PHYSICAL/CHEMICAL PROPERTY TESTS DATA GAPS: SIDS testing required: None. ENVIRONMENTAL FATE AND PATHWAY TESTS DATA GAPS: Commercial vinyl toluene consists of a mixture of meta- and para- isomers in ~60/40% ratio. Photodegradation, biodegradation, and stability in water studies of the para isomer will be used as substitute studies for vinyl toluene. No testing for vinyl toluene is proposed. ECOTOXICITY TESTS DATA GAPS: Vinyl toluene consists of a mixture of meta- and para- isomers in ~60/40% ratio. Acute toxicity to fish, acute toxicity to aquatic invertebrates, and acute toxicity to algae of the para isomer will be used as substitute studies for vinyl toluene. No testing for vinyl toluene is proposed. HEALTH EFFECTS TESTS DATA GAPS: Vinyl toluene consists of a mixture of meta- and para- isomers in ~60/40% ratio. All data gaps for vinyl toluene will be satisfied using completed studies for the para isomer. No testing for vinyl toluene is proposed.

Tier I

DATE: June 28, 2002

HPV Test Plan: Part B							
CAS No:	InfoAvail?	GLP	OECD Study	Other Study	Estim. Meth.	Acceptable?	SIDS Testing Required?
	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Physicochemical							
Melting Point	Y	N	*	Y		Y	N
Boiling Point	Y	N	*	Y		Y	N
Density[1]							
Vapor Pressure	Y	N	*	Y		Y	N
Oct: water part.coef	Y	N	*	Y		Y	N
Water solubility	Y	N	*	Y		Y	N
pKa							
Other		-	--	--	--	--	--
Environmental Fate and Pathway							
Photodeg	Y	N	*	Y		Y	N
Stability in water	Y	N	*	Y		Y	N
Monit. Data[1]							
Transp/Dist	Y	N	*		Y	Y	N
Biodeg	Y	N	*	Y		Y	N
Other		--	--	--	--	--	--
Ecotoxicology							
Acute Fish	Y	Y	Y	Y		Y	N
Acute Daph.	Y	Y	Y	Y		Y	N
Acute Algae	Y	Y	Y	Y		Y	N
Chron. Daph[2]							
Terr. Tox.[2]							
Other		--	--	--	--	--	--
Toxicology							
Acute	Y	Y	N	Y		Y	N
Rep.	Y	Y	N	Y		Y	N
DoseGenetic	Y	Y	N	Y		Y	N
Repro	Y	Y	N	Y	--	Y	N
Devel/Terat	Y	Y	N	Y		Y	N
Human Experience[2]							
Other		--	--	--	--	--	--
* Unknown [1] Not required for SIDS Base Set [2] Conditional SIDS studies							